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Assessment of Circulating Stromal Cell-Derived Factor (SDF)-1 as Prognostic Marker of Diabetes-Induced Tubular Atrophy

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Abstract

Stromal cell derived factor (SDF)-1 is a unique pleiotropic chemokine that mediates several biological and pathological functions through binding with its cognate receptor CXCR4. The SDF-1/CXCR4 axis typically exhibits a nephroprotective role, where it largely contributes to renal development and homeostasis. However, it was also reported to play an essential regulatory role in the initiation and development of diabetic kidney disease (DKD); yet, whether it is renoprotective or detrimental remains unclear. This study aimed to assess the prognostic capacity of circulating stromal cell-derived factor (SDF)-1 for diabetes-induced tubular atrophy. Thirty male Sprague Dawley rats (200-250gm) were allocated into diabetic (n=20) and sham (n=10) groups. Diabetes was induced by a single dose of 65 mg/kg STZ, then diabetic animals were categorized into treated group receiving 2-8U Mixtard®30 daily, and untreated group (n=10/each). Blood glucose, renal functions, circulating SDF-1, and inflammatory markers were weekly assessed, and renal expression of SDF-1/CXCR4 was profiled by real-time PCR. Insulin treatment ameliorated hyperglycemia and normalized renal functions and inflammation, while the untreated animals exhibited the typical course of hyperglycemia-associated nephropathy. Circulating SDF-1 levels were significantly lower in both diabetic groups than in sham (P<0.05), but persistently higher in the untreated animals (P<0.0001). Renal SDF-1/CXCR4 expression was 1.51, 2.11, and 2.76, 1.45 fold higher in untreated animals relative to non-diabetic and treated animals; respectively. ROC curve analyses of plasma/urinary SDF-1 in diabetic groups showed AUC of 0.9568 and 0.9793; respectively with cut-off values 72.78 pg/mL and 0.7182 pg/mg Cr; respectively. Both thresholds showed predictive potentials with 81.82-83.33% sensitivity and 100% specificity. In conclusion, plasma/urinary SDF-1 levels showed considerable prognostic potential for early tubular atrophy during DKD.

Keywords: SDF-1; CXCR4; diabetic kidney disease; type 1 diabetes millitus; tubular dialation; tubular atrophy; renal inflammation.

1. Introduction

Type I diabetes mellitus (T1DM) is an autoimmune disorder associated with impaired glucose homeostasis as a result of selective destruction/ or dysfunction of insulin-producing β-cells by the infiltration of T-cells into the pancreatic Langerhans islets [1]. Several elements of the immune system are implicated in the pathogenesis of T1DM, most predominantly SDF-1 (CXCL12); a member of low molecular weight chemokines that mediate recruitment and migration of autoreactive T-cells into the β-islets, leading to the pancreatic inflammation or "insulitis" [2]. SDF-1 was first described as a pre-B cell growth-stimulating factor in 1994 and was then designated as "stromal cell-derived factor" for its continuous expression in stromal cells of the bone marrow [3]. The SDF-1 chemokine has a unique pleiotropic nature, where it mediates several biological and pathological functions through binding with its cognate receptor CXCR4 [4]. The implication of the SDF-1/CXCR4 axis in the pathophysiology of diabetes and its complications has been controversial, where it was proposed that SDF-1 disturbs the balance of T-cells in favor of autoreactive T cells by retaining the regulatory T-cells (T_{reg}) in the bone marrow, which intensifies the disease progression, hence SDF-1 blockade would inhibit/delay insulitis and diabetes initiation [5]. On the other hand, a recent study proposed that the SDF-1/CXCR4 axis inhibits the dedifferentiation of islet β-cells in diabetes and accordingly restrains the disease progression [6].

SDF1/CXCR4 axis also plays an essential regulatory role in the initiation and development of diabetic kidney disease (DKD); however, whether it is renoprotective or detrimental remains dialectical [7].

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SDF-1 is reported to be expressed in the majority of renal cells, and to contribute to renal development especially vasculature formation [8]. The consensus notion about the SDF-1/CXCR4 axis is that it exerts a renoprotective effect during DKD, where it promotes the survival of renal podocytes and thusly maintains the glomerular structure; reduces glucose-induced mesangial expansion and tubular apoptosis [9]. However, another study reported that SDF-1 expressed in renal podocytes promoted glomerulosclerosis and proteinuria in type 2 diabetic mice model, and the blockade of expression efficiently halted the progressive proteinuria and glomerulosclerosis [10]. Also, the SDF-1 cognate receptor CXCR4 is expressed on the surface of several immune cells including B and T lymphocytes, which might contribute to the recruitment and migration of inflammatory cells and accordingly intensifies renal inflammation in DKD [11]. Altogether, the SDF-1/CXCR4 axis plays a dual complex effect during DKD onset that deserves further attention. In this study, we aimed to unfold such complexity seeking the determinant threshold between the renoprotective and harmful roles of SDF-1, and assess it as a potential prognostic marker from tubular degeneration during the early DKD onset**.**

2. Experimental

2.1 Approval of the Ethical Committee

Experimental procedures were approved by the Medical Ethical Committee of the National Research Centre in Egypt (Approval No: 44410112021) in strict accordance with ARRIVE Guidelines 2.0 for reporting animal research, and the National Institute of Health Guide for Care and Use of Laboratory Animals (NIH Publication no. 85–23, revised 2011) and the UK Animals (Scientific Procedures) Act 1986.

2.2 Animals

The study was conducted at the animal house of National Research Centre; Dokki-Giza; Egypt, Before the experiment, thirty 7-8 weeks old male Sprague Dawley rats (200-250 gm) were acclimated for seven days under constant environmental conditions and a 12: 12-h light/dark cycle (lights on at 7:00 am) at controlled room temperature (22 ± 2 °C). Animals were allowed free access to standard rat pellets. Food and water was daily weighed/ measured before serving and the next day, and weight/volume difference in each group represented the mean consumption per group., and the animal weights were weekly recorded. Animals were monitored by a blinded veterinarian to assess signs of fatigue and general health conditions, besides animal mortality on daily basis.

Serum Creatinine (µmol/L)	**Equation**
< 52	**eGFR (µL/min) = 880 × $W^{0.695}$ × $C^{-0.66}$ × $U^{-0.391}$**
> 52	**eGFR (µL/min) = 5862 × $W^{0.695}$ × $C^{-1.15}$ × $U^{-0.391}$**

2.3 Induction of Diabetes Mellitus

Diabetes mellitus (DM) was induced with a single high dose of 65 mg/kg STZ in 0.1 mole/L citrate buffer used as a single i.p dose to induce diabetes in 12 h-fasting rats (*n*= 20), while the sham group (*n*= 10) received only the vehicle. To limit early mortality resulting from insulin release of damaged pancreatic islets, all STZ-treated animals were intraperitoneally injected with glucagon 5µg/kg **[12]**. Fasting blood glucose (FBG) was estimated after three and seven days of STZ-injections, and animals with FBG ≥ 280 mg/dL were considered diabetic and subdivided into two weight-matched groups (*n*=10); one group was left untreated (DM untreated), while the other group received 2-8 U/daily subcutaneous dose of Mixtard®30 (long-acting; 100 IU/ml; Insulin human (rDNA) + Insulin Protophane Protamine) depending on the blood glucose levels (DM-sc insulin). If the blood glucose level was 300-400, 400-500, or > 550 mg/dL, diabetic rats were given 2, 4 or 8 units of insulin/day; respectively **[13]**. After the completion of the experimental period, animals were ether-anesthetized and sacrificed by cervical dislocation. For renal pathology studies, kidneys were dissected and rinsed with cold isotonic saline and then weighed to determine kidney hypertrophy index (Ki) by dividing the kidney wet weight by the body weight in each animal **[14]**.

2.4 Classical Serum and Urinary Biochemical Analyses

Biochemical assessments were carried out using *Biodiagnostic* kits (Biogamma, Stanbio, West Germany) according to the user manual. Following the previous protocol of **Ahmed *et al.*, [18]**, fasting blood glucose levels were estimated in diabetic rats on days 0, 7, 14, 21, and 28th using a glucose oxidase-based commercial glucometer (Accu-Chek Active, Roche Diagnostic). Renal functions were weekly monitored in terms of urine volume; blood urea (Cat# UR 21 10); plasma/urinary creatinine (Cat# CR 12 50), and urinary albumin (Cat# AB 10 10). Depending on plasma levels of creatinine, the estimated glomerular filtration rate (eGFR) was calculated according to **Besseling *et al*. [15]** in each animal using one of the following equations; where W: body weight; C: serum creatinine, U: blood urea.

2.5 Immunological Studies and Assessment of Renal Redox Status

Plasma levels of IL-1ß (Cat# SL0402Ra); TNF-α (Cat# SL0722Ra); kidney injury molecule (KIM)-1 (Cat# SL0433Ra); matrix metalloproteinase (MMP)-9 (Cat# SL0490Ra), and SDF-1 (Cat# SL0661Ra) were estimated using the corresponding Instant Rat ELISA kits (SunLong Co., Ltd- China) according to the manufacturer instructions. Urinary levels of SDF-1 were also estimated and normalized to concurrent urinary creatinine concentrations at each time point. The absorbance was measured at 450 nm and the cut-off value considered a positive response was established as twice the mean OD_{450} of the negative control. The nitric oxide production in kidney tissues and renal levels of reduced glutathione (GSH); lipid peroxidation (MDA), and activity of catalase and glutathione-s-transferase antioxidant enzymes were biochemically assessed using the corresponding kits of *Biodiagnostic* (Biogamma, Stanbio, West Germany) according to the user manual.

2.6 Real-time PCR

Gene expression of SDF-1 and its cognate receptor CXCR4 was profiled in frozen renal tissue samples preserved in RNAlater (Thermofisher Scientific, USA) directly after animal sacrifice by real-time PCR. First, total RNA was extracted from renal tissues using PureLink™ RNA Mini Kit **(Invetrogen™, USA)** according to the user manual. The quantity and quality of extracted RNA were spectrophotometrically evaluated as an $OD_{260/280}$ ratio >1.5, while the integrity was checked on 1% agarose gel premixed with 0.3% ethidium bromide. Complementary DNA (cDNA) was synthesized from 1µg of RNA template using Maxima Reverse Transcriptase (Thermofisher Scientific, USA) according to the manufacturer's protocol. According to **Siddiqi *et al*., [16]**, cDNA was used to amplify SDF-1 and CXCR4 genes besides GAPDH as housekeeping gene with the following primers:
SDF-1 F: gctctgcatcagtgacggtaag, SDF-1
R: tggcgacatggctctcaaa, CXCR4
F: atcatctccaagctgtcacactcc; CXCR4
R: gtgatggagatccacttgtgcac; GAPDH
F: accacagtccatgccatcac, GAPDH
R: tccaccaccctgttgctgta. Cycling conditions included 95˚C for 1 min; annealing temperature of respective primer for 45 s; extension at 72˚C for 1 min; final extension at 72˚C for 10 min; for 35 cycles. The melting curves were analysed to confirm the presence of specific amplification and the absence of primer dimers. Data were analysed by the comparative threshold cycle (ΔΔCt) method, and normalization was performed using the geometric mean of the GAPDH housekeeping gene.

2.7 Histopathological Assessments

For histopathological studies, the collected kidney specimens were fixed in 10% phosphate-buffered neutral formalin. Twenty-four hours after formalin fixation, specimens were routinely washed, dehydrated in serial dilutions of ethanol, cleared in xylene, and lastly embedded in paraffin. Paraffin blocks were sectioned at 4µm, and the obtained sections were stained with hematoxylin and eosin (H&E) according to the method described by **Suvarna and Layton [17]**. The severity of renal injury were assigned a quantitative score, where 0= no abnormality noted, 1= slight (minimal, <25%), 2= mild (25%-50%), 3= moderate (>50%), and 4= severe (>75%).

2.8 Statistical Analysis

Numerical data were expressed as mean ± SD in each group. Statistical analysis of all data was performed using GraphPad Prism version 9.0.2 (GraphPad, San Diego, CA). Numerical data were analyzed by unpaired parametric t-test and/or one-way analysis of variance (ANOVA) followed by Turkey's multiple comparison test. The correlation matrix was analyzed using Pearson correlation followed by linear regression tests. Two-tailed *P* values< 0.05 were considered statistically significant. Receiver operating characteristic (ROC) curve was used to calculate the appropriate threshold for prediction of prognostic threshold of investigated biomarkers using the values obtained in insulin-treated animals as control versus those of the untreated, followed by Fisher's exact test to assess the predictive capacity; sensitivity, and specificity of the calculated cut-off values.

3. Results

3.1 Changes in body weight, glycemic load, food consumption, and water intake in diabetic animals

Persistent hyperglycaemia was successfully induced in all STZ-treated animals as confirmed by fasting blood glucose estimations three days and one week after induction. No mortality was recorded in sham and insulin-treated groups, while three animals of the untreated diabetic animals (30%) died during the study period. The non-diabetic animals maintained a consistent blood glucose level throughout the study period, and they significantly gained weight (*P*= 0.0001) compared to the initial, while the untreated diabetic animals (DM-untreated) showed the classical signs of diabetes including polydipsia reflected as increased water intake; hyper-urination, and fatigue. The progressive hyperglycaemia and weight loss were also more pronounced in the DM-untreated group compared to the insulin-treated (DM-sc insulin) and sham groups [$F_{(2, 138)}$ = 14.69, *P*<0.0001] and [$F_{(2, 138)}$ = 102.8, *P*<0.0001];

respectively. At the end of the study, the terminal fasting blood glucose (FBG) and body weight were 6.427 ± 1.144mmol/L and -88.61 ± 3.581gm; respectively compared to the initial (Fig.1A, B). Insulin treatment has significantly alleviated the blood glucose; food consumption, and water intake (Fig.1C, D) from the 1st week of treatment, where it showed 37.98, 11.038, and 32.6% lower FBG, food consumption, and water intake; respectively comparing to the untreated group. On the other hand, the effect of insulin treatment on the weight loss was observed starting from the 3rd week, when the treated animals reached a terminal mean body weight of -1.127 ± 2.622compared to the initial. No statistical difference in food intake was found between the untreated diabetic group and the sham, while the insulin-treated group had a significantly lower food amount relative to both [$F_{(2, 138)}$ = 6.694, *P*= 0.0017]. Despite the insulin treatment, both diabetic groups showed a significantly higher water intake relative to sham at all time points [$F_{(2, 138)}$ = 58.28, *P*<0.0001] (Fig. 1D).

3.2 The impact of progressive hyperglycemia on renal functional and structural markers Comparing to non-diabetic and insulin-treated animals, the untreated animals had significantly higher weekly urine volume [$F_{(2, 138)}$ = 50.95, *P*< 0.0001] and higher levels of albuminuria [$F_{(2, 139)}$ = 22.16, *P*< 0.0001], concomitant with lower output of urinary creatinine [$F_{(2, 138)}$ = 8.439, *P*=0.0004]; eGFR [$F_{(2, 138)}$ = 11.31, *P*< 0.0001] and creatinine clearance [$F_{(2, 138)}$ = 8.807, *P*= 0.0003]. The highest peak of albuminuria was observed in the 2nd week then began to decline; however, it was still significantly higher than both sham and insulin-treated groups at the end of the experiment [$F_{(2, 24)}$ = 8.568, *P*= 0.0016]. Similarly, the plasma levels of creatinine; urea, and KIM-1 were significantly higher in the untreated animals relative to non-diabetic and insulin-treated [$F_{(2, 138)}$ = 52.66; 47.75, and 112.8; respectively, *P*< 0.0001 each] (Fig. 2). The insulin treatment has halted the regression of the renal functions starting from the 1st week, reaching convergent values to the control by the end of the study. The treated group also showed 32.06 and 73.21% lower urine volume and albuminuria levels (*P*<0.0001 each) concomitantly with 7.1, 4.29, and 2.32 fold higher eGFR (*P*= 0.0011), creatinine clearance (*P*<0.0001) and urinary creatinine (*P*<0.0001); respectively comparing to the untreated group. Plasma levels of creatinine and urea were normalized in the insulin-treated animals starting from the 1st week, where no statistical difference was observed relative to the sham group, while KIM-1 was normalized by the 4th. The treated animals ended up with 62.28, 71.85, and 95.76% lower levels of plasma creatinine; urea, and KIM-1; respectively compared with the untreated (*P*<0.0001, each). The mean kidney weight and kidney/body weight percentage of both diabetic groups were significantly lower than that of sham (*P*<0.0001 each), but no statistical difference in kidney/body weight % (Ki) was found despite the 40.51% larger kidney weight of the treated animals relative to the untreated (*P*<0.0001).

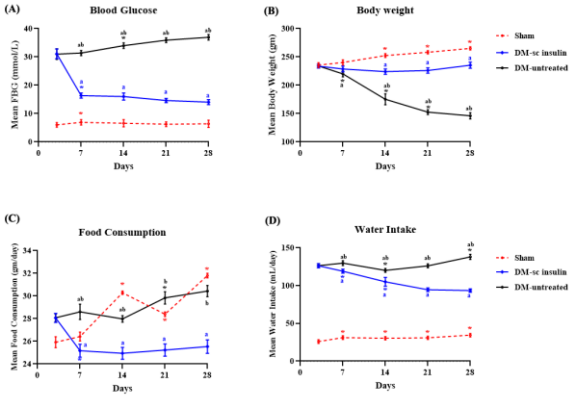
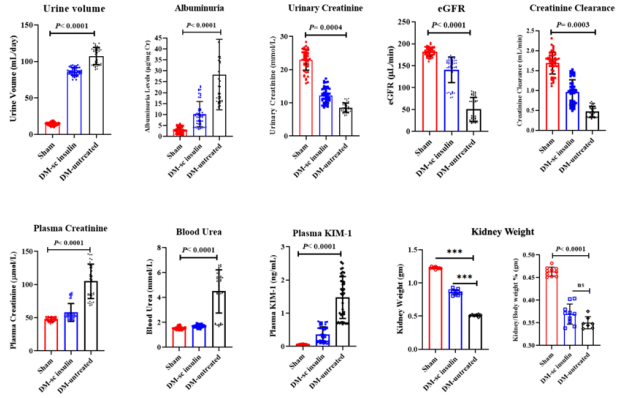


Figure 1**: Changes in blood glucose, body weight, food consumption, and water intake during the study period. Persistent hyperglycaemia (A) was generated in all STZ-treated animals, with FBG levels significantly higher in the untreated diabetic animals (*n*=7) compared to sham (*n*=10) and insulin-treated (*n*=10) along the five time points of the study [$F_{(2, 138)}$ = 14.69, *P*<0.0001]. The untreated animals also maintained a significant weight loss (B) compared to the other groups [$F_{(2, 138)}$ = 102.8,*P*<0.0001] while the insulin treatment alleviated the blood glucose and weight loss starting from the 1st and 3rd week; respectively. No statistical difference in food consumption (C) was found between sham and untreated animals, but the insulin-treated animals consumed significantly lower amounts relative to both [$F_{(2, 138)}$ = 6.694, *P*= 0.0017]. Both diabetic groups showed higher water intake (D) relative to sham at all time points [$F_{(2, 138)}$ = 58.28 , *P*<0.0001]. Non-diabetic animals maintained consistent blood glucose, weight gain, food consumption, and water intake at the expected rate. All data are represented as mean ± SD**



. Figure 2: **The effect of hyperglycaemia on renal functional and structural markers. The untreated diabetic animals showed significantly higher weekly**

urine volume [$F_{(2, 138)}$ = 50.95, P< 0.0001], albuminuria [$F_{(2, 138)}$ = 22.16, P< 0.0001], plasma creatinine , urea, and KIM-1 levels [$F_{(2, 138)}$ = 52.66; 47.75, and 112.8; respectively, P< 0.0001 each] concomitant with lower eGFR, urinary creatinine and creatinine clearance [$F_{(2, 138)}$ = 11.31, P< 0.0001; 8.439, P=0.0004, and 8.807, P= 0.0003; respectively] compared to non-diabetic and insulin-treated animals. The insulin treatment ameliorated the renal functions starting from the 1st week, where treated animals showed 32.06 and 73.21% lower urine volume and albuminuria levels (P<0.0001 each) concomitantly with 7.1, 4.29, and 2.32 fold higher eGFR (P= 0.0011), creatinine clearance (P<0.0001) and urinary creatinine (P<0.0001); respectively comparing to the untreated. Plasma creatinine, urea, and KIM-1 levels were normalized with sham by the end of the study. Kidney weights and kidney/body weight % of both diabetic groups were significantly lower compared to the non-diabetic animals (P<0.0001 each), but no statistical difference in kidney/body weight % was found between the diabetic groups although the insulin-treated had 40.51% larger kidney weight relative to the untreated (P<0.0001). All data are represented as mean ± SD

3.3 Inflammatory response and renal redox status in diabetic animals

Starting from the first week, the plasma levels of IL-1β and MMP-9 were significantly higher in the untreated animals compared to the insulin-treated [$F_{(2, 138)}$ = 21.44, and 27.01; respectively, P<0.0001 each], while no statistical difference was observed in TNF-α levels between the two groups till the 3rd week despite the higher levels in both diabetic groups relative to sham [$F_{(2, 138)}$ = 52.09, P<0.0001]. Renal production of nitric oxide in the untreated animals was estimated as 4.42 and 16.46 fold higher than insulin-treated and sham groups; respectively [$F_{(2, 21)}$ = 13.14, P= 0.0002]. Regarding the renal redox status, the untreated animals had significantly lower levels of reduced GSH [$F_{(2, 21)}$ = 2.644, P= 0.0046] concomitant with lower activity of catalase [$F_{(2, 21)}$ = 14.39, P= 0.0001] and GSH-s-transferase [$F_{(2, 21)}$ = 6.9, P= 0.0053] and higher MDA levels [$F_{(2, 21)}$ = 13.56, P= 0.0002] comparing to non-diabetic and insulin-treated animals (Fig. 3). Compared to the non-diabetic animals, the significant effect of insulin treatment on plasma levels of inflammatory markers was observed as early as one week post-treatment for IL-1β and MMP-9 which were both normalized by the 3rd week, while the reduction of TNF-α levels started from the second and maintained a significant difference at each time point till the end of the study. The plasma levels of the three pro-inflammatory markers reached final concentrations estimated as 91.65, 48.64, 60.45% lower IL-1β; TNF-α, and MMP-9 levels; respectively, whereas renal production of nitric oxide (NO) was lower by 77.41% at the end of the experiment (P<0.0001 each) compared to the untreated. The insulin treatment has also promoted the antioxidant compounds and enzymes, where treated animals had 76.55% lower MDA levels (P<0.0001) and 8.08 (P<0.0001); 1.4 (P<0.0001), and 1.44 (P= 0.0004) fold higher GSH content and catalase and GSH-s-transferase activity; respectively relative to untreated animals (Fig. 3).

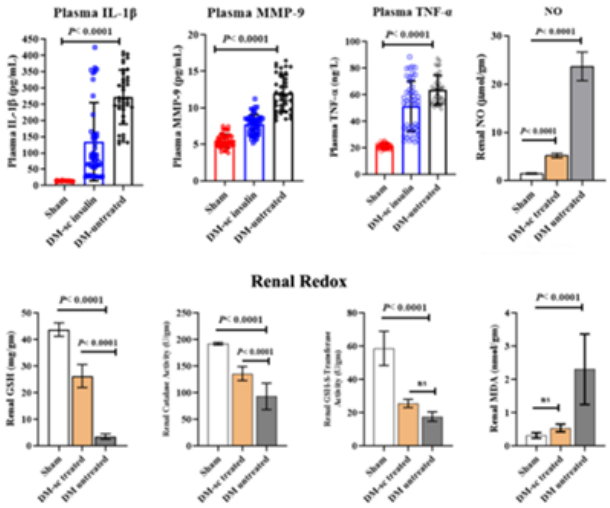


Figure 3**: Profile of inflammatory markers and renal redox status in diabetic animals. Plasma levels of IL-1β and MMP-9 were significantly higher in untreated animals starting from the 1st week relative to non-diabetic and insulin-treated animals (P<0.0001, each) despite the declined IL-1β observed in the 2nd week, while no statistical difference was observed in TNF-α levels between the diabetic groups till the 3rd. Insulin treatment normalized the plasma levels of inflammatory cytokines, reaching levels near sham control. Renal NO was 4.42 and 16.46 fold higher in untreated animals (n=4) relative to insulin-treated and non-diabetic animals; respectively [$F_{(2,21)}$ = 13.14, P= 0.0002] (n=10/each). Similarly, the dysregulated renal redox status was more pronounced in the untreated animals, were they had significantly lower activity of catalase and GSH-S-transferase enzymes [$F_{(2, 21)}$ = 14.39, P= 0.0001and 6.9, P= 0.0053; respectively]; lower GSH content [$F_{(2, 21)}$ = 2.644, P= 0.0046], and higher lipid peroxidation profile in terms of MDA levels [$F_{(2, 21)}$ = 13.56, P= 0.0002] comparing to non-diabetic and insulin-treated animals. All data are represented as mean ± SD.**

3.4 Histopathological Alterations

The histopathological assessment of renal tissues revealed normal morphology of glomerular capillaries and mesangium, and intact proximal/distal tubules with normal nuclei and cytoplasm in both sham (Fig. 4A) and insulin-treated (Fig. 4B) animal kidneys. The later; though, showed mild accumulation of hyaline droplets within the glomeruli and tubular cells, and a few distal tubules with mild degeneration and dilated lumens. In the untreated diabetic animals, the glomeruli showed mild mesangial proliferation with no basement thickening detected, and tubular atrophy with vacuolated cytoplasm and karyolitic nuclei despite the presence of intact brush borders in the proximal tubules. The

distal tubules showed dilated lumens with irregular cell morphology, and hyaline droplets were massively accumulated in both glomeruli and tubules (Fig. 4C). Renal injury scores based on histopathological assessments are listed in Table (1) (Supplementary file 1).

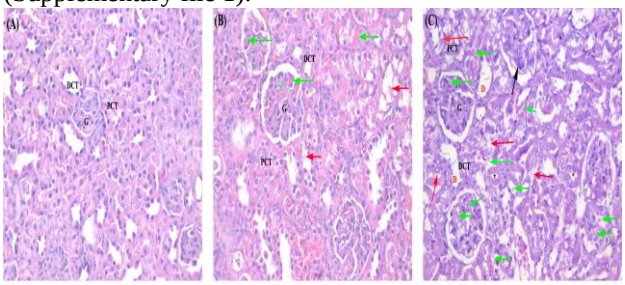


Figure 4**: Hematoxylin and eosin (H&E)-stained renal sections (×400 magnified) of sham and diabetic animals (*n*=5/group). (A): No remarkable histological alterations were observed in renal sections of the sham group. Glomerular capillaries and mesangium showed normal morphology with intact proximal and distal tubules. (B): Normal morphology of glomerular structure was observed in the insulin-treated kidneys, with few hyaline droplets (green arrows) observed in the glomeruli and proximal/distal tubules, and mild degeneration of some tubules (red arrows). (C): Tubular atrophy with vacuolated cytoplasm and karyolitic nuclei (red arrows) were observed in the sections of untreated diabetic animals. Distal tubules showed dilated lumens "D" and irregular cells, and hyaline droplets (green arrows) were massively accumulated in both glomeruli and tubules despite the intact brush border in some proximal tubules "asterisk". No mesangial expansion was detected but glomerular hypertrophy (black arrow) was observed.**

3.5 The pattern of circulating and renal expression of SDF-1 in diabetic animals

Along the five time points of the experiment, both diabetic groups showed significantly lower levels of plasma (Fig. 5A) and urinary (Fig. 5B) SDF-1 relative to the sham group [$F_{(2, 138)}$ = 3.36, *P*= 0.0376 and 11.7, *P*< 0.0001; respectively]. The non-diabetic animals maintained a constant pattern of plasma/ urinary SDF-1 levels along the study period except for a slight decline of the later observed in the 4th week, while both levels were consistently higher in the untreated animals relative to insulin-treated starting from the 1st week (*P*<0.0001, each), with no significant changes observed starting from the 1st and 2nd weeks for plasma/urinary levels; respectively. The insulin treatment normalized the urinary SDF-1 levels as early as the 1st week, showing no statistical difference relative to sham, concomitant with an abrupt decline in plasma levels before they elevate to convergent levels with those of untreated animals during the 2nd and the 3rd week then declined again by the 4th week, ending up with 20% lower levels relative to untreated animals (*P*<0.0001). Results of gene expression profiling revealed that untreated diabetic animals had 1.51 and 2.76 fold higher renal SDF-1 and CXCR4 mRNA folds compared to non-diabetic animals (*P*= 0.0046 and <0.0001; respectively), while they had 2.11 and 1.45 fold higher mRNA folds; respectively than observed in insulin-treated kidneys (*P*= 0.0001, 0.0029; respectively). Despite the absence of statistical difference, the insulin-treated animals showed lower renal SDF-1 mRNA compared to the non-diabetic; however, the renal CXCR4 mRNA fold was found to be 1.9 higher (*P*= 0.0019) (Fig. 5C).

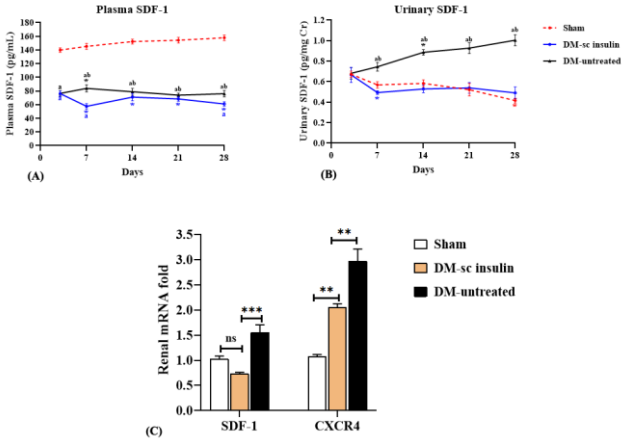


Figure 5**: The pattern of circulating SDF-1 and renal expression of SDF-1/CXCR4 in diabetic animals. Both diabetic groups showed lower levels of plasma and urinary SDF-1[$F_{(2, 138)}$ = 3.36, *P*= 0.0376 and 11.7, *P*< 0.0001; respectively] relative to non-diabetic animals along the study period. While the sham group maintained a steady pattern of circulating SDF-1, the untreated animals showed generally higher levels of both compared to insulin-treated animals (*P*<0.0001, each). No statistical difference was observed in renal SDF-1 mRNA folds between non-diabetic and insulin-treated animals (*n*=5/group), but the later showed renal CXCR4 mRNA 1.9 times higher fold (*P*= 0.0019). The untreated (*n*=5) showed 1.51, 2.11 fold higher renal SDF-1 mRNA (*P*= 0.0046 and 0.0001; respectively) and 2.76, 1.45 fold higher CXCR4 mRNA (*P*<0.0001, 0.0029; respectively) compared to sham and insulin-treated groups; respectively. All data are represented as mean ± SD.**

3.6 Correlation analyses of circulating SDF-1 levels with hyperglycemia, inflammatory and renal injury markers

The results of correlation analyses showed a different pattern in each diabetic group. In the insulin-treated group, plasma levels of SDF-1 were correlated with blood glucose (Fig. 6A.1), IL-1β (Fig. 6A.2), TNF-α (Fig. 6A.3), plasma creatinine (Fig. 6A.4), and albuminuria (Fig. 6A.5). Urinary SDF-1 was similarly correlated with blood glucose (Fig. 6A.6), IL-1β, and TNF-α (Fig. 6A.7, 8) besides KIM-1 (Fig. 6A.9), MMP-9 plasma levels (Fig. 6A.10), and plasma creatinine while inversely correlated with blood urea levels. In the untreated diabetic group, a different pattern of correlations was observed, where plasma SDF-1 levels were found to be inversely correlated with blood

glucose (Fig. 6B.1), IL-1β (Fig. 6B.2), albuminuria, and plasma creatinine (Fig. 6B.3) as well as KIM-1 (Fig. 6B.4) and MMP-9 levels (Fig. 6B.5), while they were positively correlated with TNF-α (Fig. 6B.6); eGFR (Fig. 6B.7) and creatinine clearance (Fig. 6B.8). Noteworthy, the correlation between plasma SDF-1 and eGFR initiated from the 1st week unlike other parameters that showed correlations starting from the zero time. In contrast with plasma levels, urinary SDF-1 levels were positively correlated with blood glucose (Fig. 6B.9); KIM-1 (Fig. 6B.10), and MMP-9 levels (Fig. 6B.11), as well as plasma creatinine and blood urea levels while they were inversely correlated with TNF-α (Fig. 6B.12), eGFR and creatinine clearance. Notably, a strong positive correlation between plasma and urinary SDF-1 was detected in the insulin-treated animal group (r= 0.7425, *P*<0.0001), while it was absent in the untreated. In both diabetic groups, the terminal levels of circulating SDF-1 as well as renal expression of SDF-1/CXCR4 were strongly correlated with renal SDF-1/CXCR4 mRNA folds on one hand, and with renal injury scores on the other. Pearson coefficients and *P* values of the correlation analyses between circulating/expressed SDF-1 with plasma/urine parameters and histopathological scores are listed in Tables (2) and (3) (Supplementary file). A positive correlation was also observed between urinary and plasma levels of SDF-1 in insulin-treated animals (r= 0.4875, *P*= 0.0002) (Fig. 6B.13), while none was found in the untreated.

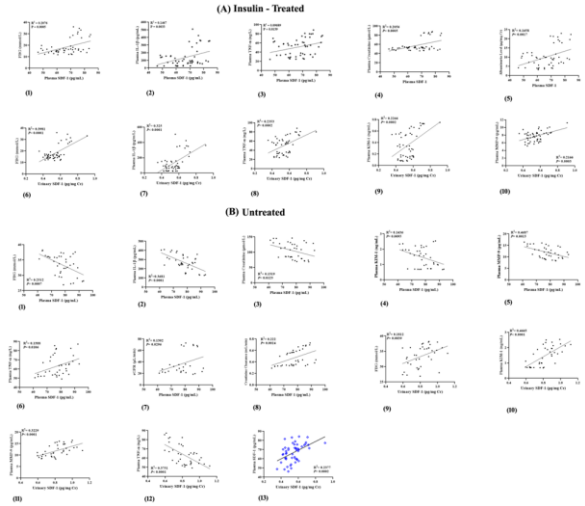


Figure 6: **(A): Linear regression of circulating SDF-1 with renal functional and structural markers in insulin-treated diabetic animals. Plasma levels of SDF-1 had positive correlations with blood glucose (r= 0.4449) (1), IL-1β (r= 0.3692) (2), TNF-α (r= 0.3346 (3), plasma creatinine (r= 0.4409) (4) and albuminuria (r= 0.4189) (5). Urinary SDF-1 levels similarly correlated with blood glucose (r= 0.6309) (6), IL-1β and TNF-α (r= 0.5701and r= 0.4661; respectively) (7) and also with KIM-1 (r= 0.4761) (8), MMP-9 (r= 0.4654) (9) and plasma creatinine (r= 0.6911). (B): Linear regression of circulating SDF-1 with renal functional and structural markers in untreated diabetic animals. Plasma levels of SDF-1 were inversely correlated with blood glucose (r= -0.5012) (1), IL-1β (r= -0.6067) (2), plasma creatinine (r= -0.3632) (3), KIM-1 (r= -0.3789) (4) and MMP-9 levels (r= -0.4687) (5), while they were positively correlated with TNF-α (r= 0.3725) (6); eGFR (r= 0.3608) (7) and creatinine clearance (r= 0.4711) (8). Urinary SDF-1 levels were positively correlated with blood glucose (r= 0.4257) (9); KIM-1 (r= 0.683) (10) and MMP-9 levels (r= 0.5683) (11), while they were inversely correlated with TNF-α (r= -0.6124) (12). Unlike the untreated animals, a positive correlation was also observed between urinary and plasma SDF-1 levels in the insulin-treated group. (r= 0.4875, *P*= 0.0002) (13).**

3.7 Prediction of Circulating SDF-1 Prognostic Threshold

While the plasma/urinary SDF-1 levels were significantly higher in untreated diabetic animals relative to insulin-treated along the study period, the prognostic potential of circulating levels for early tubular damage was assessed by receiver operating characteristic (ROC) curve, using values of circulating SDF-1 in insulin-treated animals as control against those of the untreated. For plasma SDF-1, area under curve (AUC) was 0.9568 (SEM= 0.03208, *P* <0.0001) with 95% confidence interval (CI) 0.8939 to 1.000 (Fig. 7A), while AUC for urinary SDF-1 was 0.9793 (SEM= 0.01604, *P* <0.0001) with 95% CI 0.9479 to 1.000 (Fig. 7B). The calculated threshold predicted from both curves was 72.78 pg/mL for plasma-SDF-1 with 83.33% sensitivity (95% CI: 60.78% to 94.16%) and 100% specificity (95% CI: 82.41% to 100.0%), and 0.7182 pg/mg Cr for urinary SDF-1 with 81.82% sensitivity (95% CI: 61.48% to 92.69%) and 100% specificity (95% CI: 85.13% to 100.0%). The standard renal injury markers used in this study were also subjected for ROC curve analysis and to confirm the precision of the whole experiment, and they all showed highly significant AUC values (Fig. 7 C-K) with sensitivity and specificity ranging between 82-100% and 50-90%; respectively (AUC; *P* values and predictive cut-off values with sensitivity and specificity are listed in Table (4)/ Supplementary file). Also, ROC analysis of FBG values in both diabetic groups showed a large AUC value (0.9495, *P*<0.0001). The calculated FBG cut-off predicting DKD was 26.72 mmol/L (481 pg/dL), with 100%, 82.35% sensitivity and specificity; respectively (Fig. 7L). The prognostic potential of the calculated cut-off values of circulating SDF-1 was further validated by Fisher's exact results that showed a significant predictive capacity for both plasma and urinary SDF-1 (*P* <0.0001). Plasma SDF-1 cut-off value showed 78.26% sensitivity (95% CI: 0.6443 to 0.8774) and 66.67% specificity (95% CI: 0.5155 to 0.7899) with positive and negative predictive values of 72% (95% CI: 0.5833 to 0.8253) and 73.68% (95% CI: 0.5799 to 0.8503); respectively, while the urinary SDF-1

threshold showed 84.21% sensitivity (95% CI: 0.7264 to 0.9146) and 93.55% specificity (95% CI: 0.7928 to 0.9885), with positive and negative predictive values of 96% (95% CI: 0.8654 to 0.9929) and 76.32% (95% CI: 0.6079 to 0.8701); respectively (Fig. 7M, N).

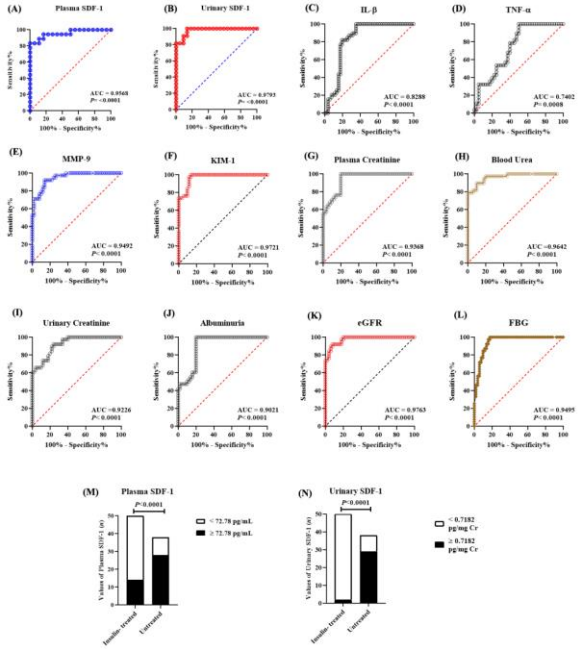


Figure 7: **Prediction of the prognostic thresholds of plasma and urinary SDF-1 by ROC curve analyses. Area under curve (AUC) for plasma SDF-1 was 0.9568 (SEM= 0.03208, *P* <0.0001) with 95% CI: 0.8939 to 1.000 (A), while AUC for urinary SDF-1 was 0.9793 (SEM= 0.01604, *P* <0.0001) with 95% CI 0.9479 to 1.000 (B). The calculated threshold predicted from both curves was 72.78 pg/mL for plasma-SDF-1 with 83.33% sensitivity (95% CI: 60.78% to 94.16%) and 100% specificity (95% CI: 82.41% to 100.0%), and 0.7182 pg/mg Cr for urinary SDF-1 with 81.82% sensitivity (95% CI: 61.48% to 92.69%) and 100% specificity (95% CI: 85.13% to 100.0%). The standard renal injury markers used in this study and FBG values were also subjected for ROC curve analysis and to confirm the precision of the whole experiment, and they all showed highly significant AUC values (C-L) with sensitivity and specificity ranging between 82-100% and 50-90%; respectively. Fisher's exact test results revealed high predictive values of the calculated SDF-1 thresholds, where plasma SDF-1 cut-off predicting DKD showed 78.26%, 66.67% sensitivity and specificity; respectively with positive and negative predictive values of 72% and 73.68%; respectively while urinary SDF-1 showed 84.21%, 93.55% sensitivity and specificity; respectively with positive and negative predictive values of 96% and 76.32%; respectively (M, N).**

4. Discussion

Although diabetic kidney disease (DKD) has always been perceived as a "glomerulopathic disorder" associated with overt microalbuminuria and declining eGFR; a growing body of evidence refers that tubular injury might precede glomerulosclerosis and even proteinuria during DKD onset [18]. While type 1 diabetes mellitus (T1DM) is an autoimmune disease prompted by the selective destruction of the pancreatic β-islets by infiltrating immune cells causing insulitis [1], several chemokines are typically involved in the disease progression and complications. Of those, the pleiotropic stromal cell-derived factor-1 (SDF-1/CXCL12) chemokine and its cognate receptor CXCR4 have been reported to exert unpredictable impacts on several pathophysiological processes in the course of T1DM [19]. On one hand, it acts as an anti-inflammatory/ immunomodulatory chemokine regulating the immune cells' differentiation and function [20], while high plasma levels of SDF-1 correlated with insulitis; nephropathy, and adipose tissue inflammation are typically reported in T2DM patients [21, 22]. SDF-1 is also expressed in most renal cells particularly stromal cells [23], and it significantly contributes to the development of renal vasculature as pro-angiogenetic chemokine [8]. In stark contrast, the SDF-1/CXCR4 axis is reportedly involved in the initiation and progression of several renal disorders, including acute renal injury; DKD, and it was recently considered a potential biomarker for lupus nephritis and renal carcinoma [7]. All of these findings have motivated us to assume that such pleiotropic behavior of SDF-1 during the DKD onset might be titer-dependent, where it might be a certain "threshold" that governs the interplay between renoprotective/pro-angiogenetic and detrimental roles, and this threshold -if present- may predict the turning point from controlled diabetes to early tubulopathy.

To test this hypothesis, we generated two diabetic models; an insulin-treated with controlled hyperglycemia, and an untreated diabetic model that underwent the normal course of DKD. In the untreated model, levels of plasma creatinine; blood urea, and eGFR showed remarkable changes as early as one week post STZ injection, which might be attributed to the transient cytotoxic effect of STZ itself on renal tissues [24] rather than the effect of hyperglycemia. In contrast to our previous report [25], a single STZ-dose was enough to induce persistent hyperglycemia in our animal model, where no spontaneous recovery was reported during the study period. This could be explained by the variation of animal strain, where Sprague Dawley rats are reported to be hypertensive, and hence more susceptible to the diabetogenic effect of STZ-impact on pancreatic β-islets [26, 27]. Despite the significant difference relative to non-diabetic animals, the insulin treatment had significantly ameliorated hyperglycemia, weight loss, hyper-urination, and

polydipsia, in addition to renal functions, eGFR, kidney redox, and inflammatory response that were normalized after two weeks of treatment. These results comply with the findings of Akbas [28] who reported the protective effect of insulin treatment on early renal pathological changes in diabetic rodents. Notably, microalbuminuria levels were significantly declined in the untreated animals after the 2nd week, reaching 26.33% lower levels than the initial despite the glomerular hyperflitration, which suggests the implication of proximal tubular reabsorption of excess albumin from the glomerular filtrate [29], particularly with the absence of enlarged glomerular basement and intact brush borders of proximal tubules as revealed by histopathological assessment.

SDF-1 is constitutively expressed in various organs, particularly the highly vascularized such as kidneys, liver, and spleen, and it is involved in several physiological functions including organs and immune cells homeostasis [30]. However, under diabetic conditions, the SDF-1 expression is significantly downregulated as a result of the impaired proliferation of stem cells associated with deficient paracrine factor release, which leads to impaired homing of endothelial progenitor cells and explains deteriorated wound healing in diabetic patients [31]. These findings of previous reports interpreted the significant reduction of circulating SDF-1 in both diabetic groups; however, they remained persistently higher in the untreated animals during the study period, which could be attributed to the higher renal SDF1/CXCR4 expression that is typically induced during renal injury to trigger homing of progenitor cells to injured sites [11]. In contrast, despite the down-regulation of SDF-1 expression in renal tissues of insulin-treated animals comparing to those of sham and untreated groups, the expression of the cognate receptor was significantly upregulated, suggesting the implication of glucose-dependent upregulation of the transcription factor HIF-α that reportedly regulates CXCR4 expression in diabetic kidneys [32, 33].

The pattern of correlations between circulating SDF-1 with blood glucose, inflammatory markers, renal functions, and redox status in diabetic groups revealed a differential mechanism of SDF-1 action in both controlled and untreated diabetic animals. In the untreated animals, the progressive hyperglycemia levels were strongly correlated with plasma MMP-9 levels, which is typical of the dysregulated redox status during diabetes that would trigger the expression of the redox-sensitive MMP-9 [34]. This would also explain the inverse correlation found between blood glucose and plasma SDF-1 levels given the catalytic activity of MMP-9 that was reported to cleave SDF-1 or at least interfere with SDF-1/CXCR4 signal transduction crucial for maintaining cell homeostasis [35], and thusly elucidate the reason of histopathological alterations observed in the untreated animals' kidneys despite the upregulated SDF-1expression. Moreover, the strong positive correlation found between plasma MMP-9 levels and urinary SDF-1 in both diabetic groups supports this assumption. Furthermore, the plasma SDF-1 levels were found to be positively correlated with TNF-α in both diabetic groups; which is, in turn, was positively correlated with eGFR in the untreated animals, while such correlation was absent in the insulin-treated. This finding suggests possible TNF-α-mediated apoptosis [36]. Unlike the other renal injury markers that were correlated with circulating SDF-1starting from three days after STZ dosing; the correlation between eGFR with plasma SDF-1 levels was not observed until the 10th day; *i.e*, after the initiation of renal injury. Furthermore, renal expression of SDF-1 and CXCR4 also showed strong correlations with renal structural markers and injury scores in both diabetic groups.

From this correlation matrix, we can conclude that SDF-1 was intrinsically overexpressed for immunomodulation and organ homeostasis in response to hyperglycemia; however, the blood glucose level is the "shunt" that dictates which role it can play. Under controlled diabetic conditions, ROS production and inflammation were alleviated and renal injury was ultimately limited; which explains the absence of correlation between circulating SDF-1 and eGFR and interprets the significant; despite weak, positive correlation between urinary and plasma SDF-1 levels absent in the untreated animals. In this context, we claim that the FBG cut-off value calculated in this study (481 mg/dL) might represent the turning point that prompt DKD. On the other side, the progressive hyperglycemic load triggered theexpression of MMP-9 that neutralized SDF-1 activity either by cleavage or intercepting with signal transduction with its receptor essential for renal homeostasis on one hand, and it also upregulated the CXCR4 expression which seems to contribute the recruitment and migration of CXCR4-positive immune cells and accordingly perpetuates renal inflammation [7].

We further investigated the prognostic capacity of different levels of circulating SDF-1 among the diabetic groups. The ROC curve analyses showed a significantly large area under curve for both plasma/urinary SDF-1 levels (0.9568 and 0.9793; respectively, P <0.0001 each), with a wide range of 95% confidence interval (0.8939 to 1.000) and high sensitivity (81.82-83.33%) and specificity (100% each) of the predicted cut-off values. Moreover, the calculated values showed high positive and negative predictive values with sensitivity and specificity ranges of 78.26%-84.21% and 66.67%-93.55% and 95% confidence interval reaching to 98.85%. Despite the large AUC values obtained for the classic renal injury markers studied herein, none of these markers

were correlated with structural markers of renal injury in such early point of the DKD onset. Together, with their strong correlations with functional and structural markers beside the injury scores, the calculated thresholds may represent the thin line between protective and detrimental roles of SDF-1 in DKD, besides their high prognostic capacity for early tubular atrophy during the disease onset.

5. Conclusion

This study represents the first in-depth reporting of the effect of the SDF-1/CXCR4 axis on renal inflammation during DKD. Herein, we proposed an SDF-1 threshold that could elucidate the determinant point between the renoprotective and pro-inflammatory role of this pleiotropic chemokine. Notably, the cross-talk between SDF-1 and cognate receptor expressed on both renal and inflammatory cells is largely complicated, but our results showed that it is mostly orchestrated by the blood glucose levels that promoted the oxidative stress and the downstream cascade of inflammatory response that prompted the renal injury. While tubular injury seems to initiate before the typical glomerulosclerosis in DKD, the proposed predictive values of circulating SDF-1 may represent a promising biomarker for diabetes-induced tubular atrophy.

6. Competing Interests

The authors have no competing interests to declare that are relevant to the content of this article.

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9. Abbreviations: SDF-1: stromal cell-derived factor 1, DKD: diabetic kidney disease, FBG: fasting blood glucose, STZ: streptozotocin, IL: interleukin, TNF-α: tumor necrosis factor, Ki: kidney hypertrophy index, eGFR: estimated glomerular filtration rate, Cr: creatinine, CXCR4: C-X-C receptor 4, GAPDH: glyceraldehyde-3-phosphate dehydrogenase, MMP-9: matrix metalloprotease-9, KIM-1: kidney injury molecule 1, i.p: intraperitoneal, sc: subcutaneous

10. References

1. Gouda W, Ismail MF, Shaker OG, Wahba E, Yousif HM, Afify M. The combined effect of ACE, TCF7L2, and PPARGC1A gene polymorphisms in diabetic nephropathy. *Egyptian Journal of Chemistry*. 60(5), 869-81, doi: https://dx.doi.org/10.21608/ejchem.2017.1335.1084 (2017).
2. Vidakovic M, Grdovic N, Dinic S, Mihailovic M, Uskokovic A, Arambasic Jovanovic J. The importance of the CXCL12/CXCR4 axis in therapeutic approaches to diabetes mellitus attenuation. *Front. immunol.* 6, 403, doi: https://dx.doi.org/10.3389%2Ffimmu.2015.00403 (2015).
3. Bleul CC, Fuhlbrigge RC, Casasnovas JM, Aiuti A, Springer TA. A highly efficacious lymphocyte chemoattractant, stromal cell-derived factor 1 (SDF-1). *J. Exp. Med*. 184(3), 1101-9, doi: https://doi.org/10.1084/jem.184.3.1101 (1996).
4. Kucia M, Jankowski K, Reca R, Wysoczynski M, Bandura L, Allendorf DJ, Zhang J, Ratajczak J, Ratajczak MZ. CXCR4–SDF-1 signalling, locomotion, chemotaxis and adhesion. *J. Mol. Histol*. 35(3), 233-45, doi: https://doi.org/10.1023/b:hijo.0000032355.66152.b8 (2004).
5. Leng Q, Nie Y, Zou Y, Chen J. Elevated CXCL12 expression in the bone marrow of NOD mice is associated with altered T cell and stem cell trafficking and diabetes development. *BMC Immunol*. 9(1), 1-2; doi: https://doi.org/10.1186/1471-2172-9-51 (2008).
6. Chen XY, Shi YX, Huang YP, Ding M, Shen QL, Li CJ, Lin JN. SDF-1 inhibits the dedifferentiation of islet β cells in hyperglycemia by up-regulating FoxO1 via binding to CXCR4. *J. Cell. Mol. Med*. 26(3), 750-63, doi: https://doi.org/10.1111/jcmm.17110 (2022).
7. Song A, Jiang A, Xiong W, Zhang C. The Role of CXCL12 in Kidney Diseases: A Friend or Foe?. *Kidney Dis. (Basel)*. 7(3), 176-85, doi: https://doi.org/10.1159/000514913 (2021).
8. Takabatake Y, Sugiyama T, Kohara H, Matsusaka T, Kurihara H, Koni PA, Nagasawa Y, Hamano T, Matsui I, Kawada N, Imai E. The CXCL12 (SDF-1)/CXCR4 axis is essential for the development of renal vasculature. *J. Am. Soc. Nephrol*. 20(8), 1714-23, doi: https://dx.doi.org/10.1681%2FASN.2008060640 (2009).
9. Takashima S, Fujita H, Fujishima H, Shimizu T, Sato T, Morii T, Tsukiyama K, Narita T, Takahashi T, Drucker DJ, Seino Y. Stromal cell-derived factor-1 is upregulated by dipeptidyl peptidase-4 inhibition and has protective roles in progressive diabetic nephropathy. *Kidney Int*. 90(4), 783-96, doi: https://doi.org/10.1016/j.kint.2016.06.012 (2016).
10. Sayyed S, Hägele H, Kulkarni OP, Endlich K, Segerer S, Eulberg D, Klussmann S, Anders HJ. Podocytes produce homeostatic chemokine stromal cell-derived factor-1/CXCL12, which contributes to glomerulosclerosis, podocyte loss, and albuminuria in a mouse model of type 2 diabetes. *Diabetologia*. 52(11), 2445-54, doi: https://doi.org/10.1007/s00125-009-1493-6 (2009).
11. Tögel F, Isaac J, Hu Z, Weiss K, Westenfelder C. Renal SDF-1 signals mobilization and homing of CXCR4-positive cells to the kidney after ischemic injury. *Kidney Int*. 67(5), 1772-84, doi: https://doi.org/10.1111/j.1523-1755.2005.00275.x (2005).

12. Dirnena-Fusini I, Åm MK, Fougner AL, Carlsen SM, Christiansen SC. Intraperitoneal, subcutaneous and intravenous glucagon delivery and subsequent glucose response in rats: a randomized controlled crossover trial. *BMJ Open Diabetes Res. Care*. 6(1), e000560, doi: https://dx.doi.org/10.1136%2Fbmjdrc-2018-000560 (2018).
13. Kuo H, Wu P, Kuo C, Chen Y. Effect of insulin on the expression of intraocular vascular endothelial growth factor in diabetic rats. *Chang Gung Med. J*. 29(6), 555, (2006).
14. Habib SL. Kidney atrophy vs hypertrophy in diabetes: which cells are involved?. *Cell Cycle*. 17(14), 1683-7, doi: https://dx.doi.org/10.1080%2F15384101.2018.1496744 (2018).
15. Besseling PJ, Pieters TT, Nguyen IT, de Bree PM, Willekes N, Dijk AH, Bovée DM, Hoorn EJ, Rookmaaker MB, Gerritsen KG, Verhaar MC. A plasma creatinine-and urea-based equation to estimate glomerular filtration rate in rats. *Am. J. Physiol. Renal Physiol*. 320(3), F518-24, doi: https://doi.org/10.1152/ajprenal.00656.2020 (2021).
16. Siddiqi FS, Chen LH, Advani SL, Thai K, Batchu SN, Alghamdi TA, White KE, Sood MM, Gibson IW, Connelly KA, Marsden PA. CXCR4 promotes renal tubular cell survival in male diabetic rats: implications for ligand inactivation in the human kidney. *Endocrinology*. 156(3), 1121-32, doi: https://doi.org/10.1210/en.2014-1650 (2015).
17. Suvarna K, Layton C. The gross room/surgical cut-up. In: Suvarna SK, Layton C, Bancroft JD, editors. Bancroft's Theory and Practice of Histological Techniques (7th Edition). Oxford: Churchill Livingstone. pp. 95-103, (2013).
18. Yang Z, Lou X, Zhang J, Nie R, Liu J, Tu P, Duan P. Association Between Early Markers of Renal Injury and Type 2 Diabetic Peripheral Neuropathy. *Diabetes Metab. Syndr. Obes*. 14, 4391; doi: https://dx.doi.org/10.2147%2FDMSO.S335283 (2021).
19. Krieger JR, Ogle ME, McFaline-Figueroa J, Segar CE, Temenoff JS, Botchwey EA. Spatially localized recruitment of anti-inflammatory monocytes by SDF-1α-releasing hydrogels enhances microvascular network remodeling. *Biomaterials*. 77, 280-90, doi: https://doi.org/10.1016/j.biomaterials.2015.10.045 (2016).
20. Alagpulinsa DA, Cao JJ, Sobell D, Poznansky MC. Harnessing CXCL12 signaling to protect and preserve functional β-cell mass and for cell replacement in type 1 diabetes. *Pharmacol. Ther*. 193, 63-74, doi: https://doi.org/10.1016/j.pharmthera.2018.08.011 (2019).
21. Lu CF, Ma JH, Su JB, Wang XQ, Liu WS, Ge XQ. Serum stromal cell-derived factor-1 levels are associated with diabetic kidney disease in type 2 diabetic patients. *Endocr. J*. 68(9), 1101-7, doi: https://doi.org/10.1507/endocrj.EJ21-0039 (2021).
22. Liu WS, Hua LY, Zhu SX, Xu F, Wang XQ, Lu CF, Su JB, Qi F. Association of serum stromal cell-derived factor-1 levels with EZSCAN score and its derived indicators in patients with type 2 diabetes. *Endocr. Connect*. 1, EC-21-0629, doi: https://doi.org/10.1530/EC-21-0629 (2022).
23. Gröne HJ, Cohen CD, Gröne E, Schmidt C, Kretzler M, Schlöndorff D, Nelson PJ. Spatial and temporally restricted expression of chemokines and chemokine receptors in the developing human kidney. *J. Am. Soc. Nephrol*. 13(4), 957-67, doi: https://doi.org/10.1681/asn.v134957 (2002).
24. Eleazu CO, Iroaganachi M, Eleazu KC. Ameliorative potentials of cocoyam (Colocasia esculenta L.) and unripe plantain (Musa paradisiaca L.) on the relative tissue weights of streptozotocin-induced diabetic rats. *J Diabetes Res*. 2013:160964. doi: https://doi.org/10.1155/2013/160964 (2013).
25. A. Ahmed S, M. Aziz W, E. Shaker S, Fayed D, Shawky H. Urinary transferrin and proinflammatory markers predict the earliest diabetic nephropathy onset. *Biomarkers*. 27(2), 178-187, doi: https://doi.org/10.1080/1354750x.2021.2023639 (2021).
26. Clements KM, Wainwright PE. Spontaneously hypertensive, Wistar-Kyoto and Sprague-Dawley rats differ in performance on a win-shift task in the water radial arm maze. *Behav Brain Res*. 167(2), 295-304, doi: https://doi.org/10.1016/j.bbr.2005.09.016 (2006).
27. Rocha NN. Are Wistar Rats the Most Suitable Normotensive Controls for Spontaneously Hypertensive Rats to Assess Blood Pressure and Cardiac Structure and Function?. *Int. J. Cardiovasc. Sci*. 35(2), 172-3, doi: https://doi.org/10.36660/ijcs.20210286 (2022).
28. Akbas F. Protective effect of insulin treatment on early renal changes in streptozotocin-induced diabetic rats. *Acta Endocrinol. (Buchar.)*. 14(2), 169, doi: https://doi.org/10.4183/aeb.2018.169 (2018).
29. Zeni L, Norden AG, Cancarini G, Unwin RJ. A more tubulocentric view of diabetic kidney disease. J. Nephrol. 30(6), 701-17, doi: https://doi.org/10.1007/s40620-017-0423-9 (2017).
30. Ho TK, Shiwen X, Abraham D, Tsui J, Baker D. Stromal-cell-derived factor-1 (SDF-1)/CXCL12 as potential target of therapeutic angiogenesis in critical leg ischemia. *Cardiol. Res. Pract*. 2012, 143209, doi: https://doi.org/10.1155/2012/143209 (2012).
31. Mayorga ME, Kiedrowski M, McCallinhart P, Forudi F, Ockunzzi J, Weber K, Chilian W, Penn MS, Dong F. Role of SDF-1: CXCR4 in impaired post-myocardial infarction cardiac repair in diabetes. *Stem Cells Trans. Med*. 7(1), 115-24, doi: https://dx.doi.org/10.1002%2Fsctm.17-0172 (2018).
32. Isoe T, Makino Y, Mizumoto K, Sakagami H, Fujita Y, Honjo J, Takiyama Y, Itoh H, Haneda M. High glucose activates HIF-1-mediated signal transduction in glomerular mesangial cells through a carbohydrate response element binding protein. *Kidney Int*. 78(1):48-59, doi: https://doi.org/10.1038/ki.2010.99 (2010).
33. Ding M, Cui S, Li C, Jothy S, Haase V, Steer BM, Marsden PA, Pippin J, Shankland S, Rastaldi MP, Cohen CD, Kretzler M, Quaggin SE. Loss of the tumor suppressor Vhlh leads to upregulation of Cxcr4 and rapidly progressive glomerulonephritis in mice. *Nat Med*. 12(9):1081-7. doi: https://doi.org/10.1038/nm1460 (2006).
34. Uemura S, Matsushita H, Li W, Glassford AJ, Asagami T, Lee KH, Harrison DG, Tsao PS. Diabetes mellitus enhances vascular matrix metalloproteinase activity:

role of oxidative stress. *Circ. Res*. 88(12), 1291-8, doi: https://doi.org/10.1161/hh1201.092042 (2001).

35. Jin F, Zhai Q, Qiu L, Meng H, Zou D, Wang Y, Li Q, Yu Z, Han J, Zhou B. Degradation of BM SDF-1 by MMP-9: the role in G-CSF-induced hematopoietic stem/progenitor cell mobilization. *Bone marrow Transplant*. 42(9), 581-8, doi: https://doi.org/10.1038/bmt.2008.222 (2008).
36. Jarrah AA, Schwarskopf M, Wang ER, LaRocca T, Dhume A, Zhang S, Hadri L, Hajjar RJ, Schecter AD, Tarzami ST. SDF-1 induces TNF-mediated apoptosis in cardiac myocytes. *Apoptosis*. 23(1), 79-91, doi: https://doi.org/10.1007/s10495-017-1438-3 (2018).